

Serum IgE antibodies to *Trichophyton* in patients with urticaria, angioedema, asthma, and rhinitis: Development of a radioallergosorbent test

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A series of patients was identified who demonstrated immediate positive skin tests to intradermal *Trichophyton* extract. These skin responses did not correlate with other fungal skin tests and were present both in atopic and nonatopic patients. The individuals demonstrating positive immediate skin tests included patients with urticaria, angioedema, asthma, and/or rhinitis, as well as five of 34 normal control subjects. Most skin test positive individuals had a history of local fungal infection and clinical signs suggestive of fungal infection. By use of *Trichophyton tonsurans* extract linked to Sepharose as the immunosorbent, it was possible to measure IgE antibodies in 26/30 sera from skin test positive individuals. With strongly positive sera, RAST bound up to 30% of the radiolabeled anti-IgE added. The results confirm that most skin test positive individuals have IgE-mediated hypersensitivity to *Trichophyton*. These observations support the older view that absorption of dermatophyte allergen through the skin should be considered as a possible cause of allergic disease. (J ALLERGY CLIN IMMUNOL 1987;79:40-5.)

The possible role of immediate hypersensitivity to *Trichophyton* allergens in diseases such as urticaria, rhinitis, and asthma was widely discussed during the 1930s.¹ Two articles^{2,3} in the last two decades have suggested a role for *Trichophyton* antigens in chronic urticaria. It has also been reported that podiatrists become immune to *Trichophyton* and develop symptoms on exposure to the dust from infected toenails.⁴ There have been several in vitro studies on the immune response to *Trichophyton* antigens.⁴⁻⁷ These studies have suggested that the nature of the immune response and the atopic state of the individual can alter the clinical course of the fungal infection but have not related these infections to any other disease.^{5,6} Surprisingly, there have been very few articles on the in vitro measurement of IgE antibody to *Trichophyton*. However, it has been reported that IgE antibody to fungal antigens can be measured in the serum of pa-

Abbreviations used

BBS:	Borate-buffered saline, pH 8.0
CRIE:	Crossed radioimmunolectrophoresis
SAS:	Saturated ammonium sulfate
OD:	Optical density

tients from New Guinea who have severe tinea imbricata.⁸ Those results were obtained by use of an ELISA assay on microtiter plates.⁸ There has also been a study from Denmark demonstrating that IgE antibody to *T. rubrum* can be detected by use of CRIE.⁹ However, a RAST is not currently available, and there are no studies of measurements of IgE antibody to *Trichophyton* in sera from patients with allergic diseases and fungal infection.

We became interested in immediate hypersensitivity to *Trichophyton* because we found several patients who had urticaria or recurrent angioedema associated with positive immediate skin tests to intradermal *Trichophyton* and who also had signs of fungal infection of their nails, feet, and/or groins.¹⁰ Further skin testing revealed positive skin tests in patients with asthma, some of whom had no other positive skin tests and also had signs of fungal infection. We report here the development of a RAST with *T. tonsurans* extract

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TABLE I. Effects of species of *Trichophyton*, partial fractionation of extracts, and immunosorbent on RAST for *Trichophyton*-specific IgE antibodies*

Serum	Cellulose discs						Sephadex	
	<i>T. tonsurans</i>		<i>T. rubrum</i>		<i>Trich mix†</i>		<i>T. tonsurans</i>	<i>Trich mix</i>
	Whole extract	60% to 90% SAS	Whole extract	60% to 90% SAS	Whole extract	60% to 90% SAS	Whole extract‡	Whole extract
HD.	9.1	11.8	3.4	2.7	7.6	5.7	25.7	23.3
RJ.	1.8	2.6	0.9	1.0	1.2	1.1	4.2	5.2
SS.	1.5	1.2	1.5	1.3	1.6	2.6	22.5	22.4
Controls	0.63	0.66	0.67	0.60	0.64	0.63	0.48	0.51
n = 4								

*Values are the mean of duplicate values for percent radioactive counts bound. All sera were assayed undiluted with 0.1 ml. For all other reagents in the assay, 50% horse serum was used as diluent. In separate experiments with 1% bovine serum albumin as a diluent, results for the same four skin test negative control subjects varied from 1.3% to 2.6% of counts bound. Extracts and 60% to 90% SAS fractions were dialyzed for 2 days against four changes of BBS.

†Mixed extract containing *T. tonsurans*, *T. rubrum*, and *T. mentagrophytes*.

‡In a separate experiment, values obtained with 60% to 90% SAS fraction on Sephadex were very similar.

linked to Sephadex as the immunosorbent. The results demonstrate that IgE antibody to *Trichophyton* can be detected in serum from most individuals with strong positive immediate skin tests. These results are not restricted to patients with urticaria or asthma, since we have also found positive results among normal control subjects. Nonetheless, we believe our results suggest that the role of *Trichophyton* as an "intrinsic" allergen should be reconsidered.

PATIENTS AND METHODS

Sera were obtained from patients evaluated in the University of Virginia Allergy Clinic, including 27 patients with urticaria or recurrent angioedema, 39 patients with asthma, and seven patients with rhinitis. The patients with asthma included 15 patients who had demonstrated strongly positive skin tests to *Trichophyton* and 21 who had negative or weakly positive skin tests to *Trichophyton*. The patients with urticaria or recurrent angioedema included eight with strongly positive skin tests and 19 patients with negative or weakly positive skin tests to *Trichophyton*. Two of the seven patients with rhinitis had positive skin tests to *Trichophyton*. The patients with negative skin tests were selected to include patients with strong positive skin tests to other allergens. Thirty-four volunteers who were employed in the hospital were skin tested with both mite and *Trichophyton* extracts, answered a brief questionnaire, and provided a serum sample. In addition, 61 sera were available from patients with asthma treated in the emergency room. All procedures involving patients were approved by the University of Virginia Human Investigation Committee.

Extracts and skin testing

Skin testing was carried out with a mixture of *T. tonsurans*, *T. rubrum*, and *T. mentagrophytes* 1:10 w/v (Hol-

lister-Stier, Spokane, Wash.). This extract was used undiluted for prick tests and 0.02 ml of 1:20 dilution (i.e., 1:200 w/v) was used for intradermal skin testing. Skin tests were graded 2+ = >5 mm wheal on prick test or >8 by 8 mm on intradermal tests and 1+ = >5 by 5 mm wheal on intradermal skin test. Patients were also skin tested with extracts of *Alternaria*, *Cladosporium*, and *Aspergillus*, as well as a range of other inhalant allergens as part of their routine evaluation. Skin tests to these three fungi were carried out by use of prick test and intradermal technique, as with *Trichophyton* and evaluated in the same way.

Cultures of *T. tonsurans* and *T. rubrum* that had been filtered from the fluid medium, rinsed, and freeze-dried were kindly provided by Hollister-Stier. Cultures were extracted with BBS, pH 8.0, at 1:7 w/v overnight in the cold, centrifuged to remove debris, and dialyzed. The mixed *Trichophyton* extract was also dialyzed against BBS. Extracts were compared according to their OD at 280 nm; 1 ml of extract with an OD of 1.0 equaled 1 OD unit. In some experiments extracts were fractionated with SAS by precipitation with 60% SAS followed by precipitation at 90% SAS. Both the 60% SAS precipitate and the 60% to 90% SAS precipitate were dialyzed against three changes of BBS.

RAST for IgE antibodies to *Trichophyton* by use of Sephadex

Dialyzed extracts were linked to CNBr-activated Sephadex (Pharmacia fine chemicals, Piscataway, N. J.) at a ratio of 10 OD units per gram of dry gel.¹¹ The gels were suspended to 6 ml/gr by use of 0.1 mol/L NaHCO₃, pH 9.0, as coupling buffer and rotated at 4° C overnight. The Sephadex was then washed three times with bicarbonate buffer, incubated with 1 mol/L of ethanolamine for 2 hours, and washed with acetate buffer, pH 4.0, three times and with BBS three times. Sephadex was suspended to a final volume of 9.0 ml/gr of dry gel.

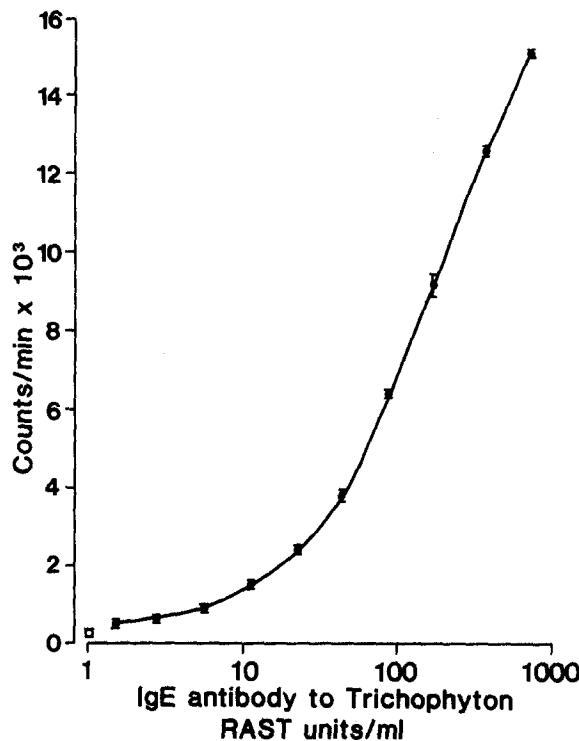


FIG. 1. Binding curve for IgE antibody to *T. tonsurans*. Serial twofold dilutions of the control serum were assayed in duplicate, mean (●), and range of duplicate values are indicated. Also presented is the mean \pm SD for duplicate assays on sera from six nonallergic control subjects (○).

For assays, 50 μ l of *Trichophyton* Sepharose suspension was incubated with 100 μ l of serum or serum diluted with 50% horse serum, and rotated overnight at room temperature in 12 mm by 75 mm plastic tubes. After washing three times with BBS, 125 I-labeled goat anti-IgE (\sim 100,000 cpm) was added in 200 μ l of 50% horse serum¹² and again rotated overnight at room temperature. Finally, the Sepharose was washed three times and counted in a Micromedic (Micromedic Systems, Horsham, Pa.) 4-channel automatic gamma counter. Six sera from nonallergic control subjects were assayed in duplicate in parallel with each assay, and values >2 SD greater than the mean for the control sera were regarded as positive. However, in assaying sera from allergic patients, it became evident that nonspecific binding was higher with sera from patients with high total IgE. Background binding was assessed in a series of skin test negative patients; background was $0.48 \pm 0.07\%$ for 28 sera with <100 IU of IgE per milliliter; $0.66 \pm 0.18\%$ for 30 sera with 100 to 1000 IU of IgE per milliliter, and $1.58 \pm 0.95\%$ for nine sera with >1000 IU of IgE per milliliter. All sera were assayed for total IgE, and relevant background was subtracted before calculating results.

Paper disc RAST

Cellulose discs were made from Whatman No. 1 filter paper and activated with CNBr with standard techniques.¹³

Discs were coated individually with *Trichophyton* extract diluted 1:20 in 0.1 mol/L NaHCO₃, 100 μ l per disc, overnight. *Dermatophagoides pteronyssinus* RAST was carried out as described previously.¹³

RESULTS

RAST for IgE antibody to *Trichophyton*

Initial experiments with *Trichophyton* extract linked to cellulose discs produced very poor binding, maximum binding 4% of added radioactivity. These results were improved either by extensive dialysis of extracts or by taking a 60% to 90% SAS precipitate (Table I). However, with dialyzed extract linked to Sepharose 4B, we obtained better binding (up to 30% of added anti-IgE bound) (Table I). With up to 3% bovine serum albumin in the RAST diluent, the nonspecific background was very high (up to 2.5%). Marked reduction in background was obtained by use of 50% horse serum as diluent in all phases of the assay.¹² Nonetheless, background was higher for sera with high total IgE, and relevant background values were subtracted (see Methods). Both binding phases of the assay were found to require rotation of the tubes, and the assay elicited most consistent results with prolonged incubation periods, i.e., greater than or equal to 12 hours. In preliminary experiments with both cellulose discs and Sepharose, we found better binding to *T. tonsurans* and to *Trichophyton* mixture than to *T. rubrum* (these results are presented in part in Table I). One hundred sera were assayed both against *T. tonsurans* and *Trichophyton* mixture on Sepharose. The results were closely comparable with an overall correlation coefficient of $r = 0.96$ ($p < 0.001$) (data not presented). For the routine assay, *T. tonsurans* on Sepharose was used, and quantitation was obtained by assaying serial twofold dilutions of a strong positive serum that was arbitrarily allotted 1000 RAST units of IgE antibody to *T. tonsurans* (Fig. 1). Each test serum was assayed at two dilutions. Results were interpolated from the control curve, and mean intra-assay variation for the two dilutions was $\pm 16\%$ of the mean. Repeated assays on 30 sera demonstrated a coefficient of variation of 27%.

Several approaches were used to assess the specificity of the assay. The results with different species of *Trichophyton* suggest that there is extensive cross-reactivity among them. All the sera were assayed for IgE antibody to *D. pteronyssinus*, and these results demonstrated no correlation with *Trichophyton* results (Fig. 2). We also examined skin test records on 45 of the patients. Although many of the patients had other positive skin tests, there was no consistent pattern. In particular 16/24 of the patients with strong positive skin tests to *Trichophyton* had no other com-

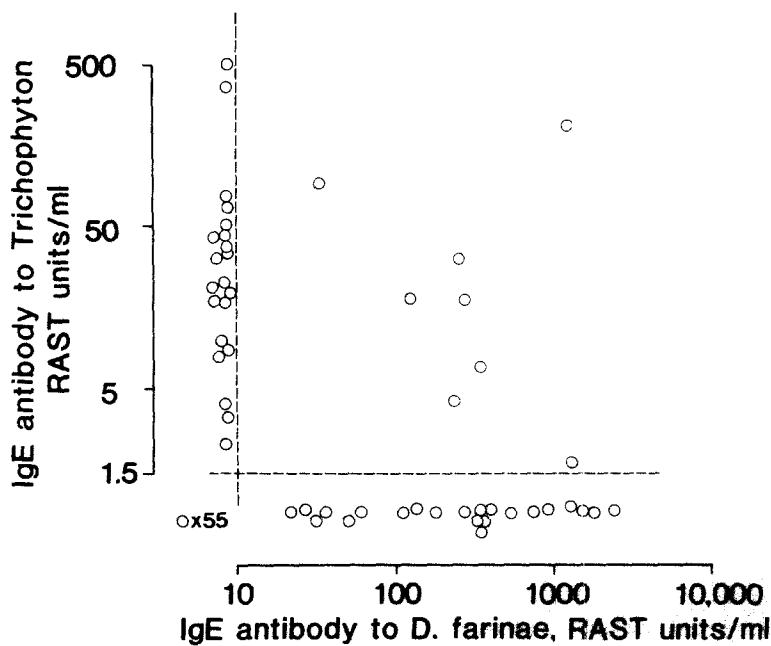


FIG. 2. IgE antibody to *Trichophyton* and IgE antibody to *D. farinae* assayed in 106 sera. Fifty-five sera that demonstrated no IgE antibody to either allergen are indicated (0×55) below the lower limit for each assay. There was no correlation between the results for the two assays ($r = -0.16$) ($p > 0.2$).

parable (i.e., 2+) skin test to the other fungi tested. These results suggest that positive skin tests to other fungi are likely to represent concordant sensitivity rather than cross-reactivity. Finally, we assayed 61 sera for IgE antibody to *Alternaria*. Five sera in that group were positive for *Alternaria* alone, three sera to *Trichophyton* alone, and four sera were positive to both. Again the results suggest that the examples of dual sensitivity are likely to represent concordant sensitivity rather than cross-reactivity.

IgE antibodies to *Trichophyton* in sera from patients with angioedema, urticaria, asthma, or rhinitis

Among 27 patients with chronic urticaria and/or recurrent angioedema, we found eight patients with strong positive skin tests to *Trichophyton* and six additional patients with weaker skin reactions. Nine of these patients had serum IgE antibody to *Trichophyton*. Eight had strong positive skin tests plus one who demonstrated a 6 by 6 mm wheal (Fig. 3). Four of these patients had no other strong positive skin tests. The others demonstrated various positive skin tests including mite (two), ragweed (one), wheat (one), and *Cladosporium* (*Hormodendrum*) (one).

Strong positive skin tests to *Trichophyton* were not very common among patients with asthma. However, we have identified 15 patients with 2+ skin tests of

approximately 120 who were skin tested. In sera from 12 of those 15 patients, we found detectable serum IgE antibody to *Trichophyton* with values ranging from 10 to 500 RAST units per milliliter (Fig. 3). Of the 12 patients with asthma who had serum IgE antibody to *Trichophyton*, four also had IgE antibody to *D. pteronyssinus*, whereas eight patients had none. In addition to clinic patients, we assayed sera from 61 patients with asthma observed in the emergency room and elicited positive results from seven sera (range 2.3 to 35 *Trichophyton* RAST units per milliliter). However, of these seven sera, six had multiple IgE antibodies to other allergens as assessed by RAST (data not presented). Indeed, the one patient whose serum contained IgE antibody to *Trichophyton* (35 U/ μ l) but was otherwise negative had already been identified as *Trichophyton* sensitive during a clinic visit and was one of the patients included in Fig. 3.

A strong positive (2+) immediate skin test was obtained in five of thirty-four individuals who worked in the hospital and were skin tested with *Trichophyton*. Of these individuals, four had detectable serum IgE antibody to *Trichophyton* (Fig. 3). All four of the control subjects who had IgE antibody to *Trichophyton* had had chronic fungal infection of the feet requiring local antifungal treatment, but none of them had had urticaria or asthma.

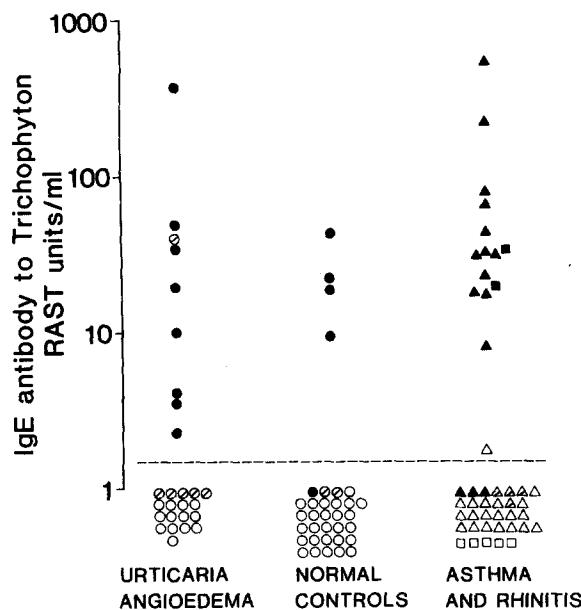


FIG. 3. IgE antibody to *T. tonsurans* in sera from patients with urticaria, angioedema, asthma, or rhinitis. Individuals with strong positive skin tests to *Trichophyton* (2+) are indicated by solid symbols (●, ▲, and ■). Individuals with weak positive skin tests (1+) are indicated by slashed symbols (○, △). Patients with asthma (▲, △) are distinguished from those with rhinitis (■, □).

DISCUSSION

Although there have been many studies of positive immediate skin tests to *Trichophyton*, there have been surprisingly few studies of in vitro assays of IgE antibody to this antigen.^{8, 9} Serum reagins were confirmed by passive transfer (Prausnitz-Küstner test) in the 1930s.^{1, 14} We could not find any published articles of positive RAST results. With cellulose discs as immunosorbent, many positive sera demonstrated very low levels of binding, but we obtained consistent results and a good correlation with positive skin tests with Sepharose. An ELISA technique for measuring IgE antibody to *Trichophyton* has been reported, which has been used on sera from patients who had the more severe fungal infection tinea imbricata.^{6, 8} The microtiter plates used for ELISA assays have relatively low capacity for antigen, and our results with paper discs suggest that optimal results with *Trichophyton* extracts require a high capacity immunosorbent. The only other technique reported for measuring IgE antibody to *Trichophyton* was CRIE.⁹ The results of CRIE are only semiquantitative, and the technique is both difficult and time-consuming compared to RAST. Indeed, results were only reported on seven sera.⁹ Interestingly, many of the patients whom we regarded as having strongly positive intradermal

skin tests to *Trichophyton* extract only demonstrated minimal responses to prick test, e.g., 3 by 3 to 4 by 4 mm wheals. At present it is difficult to answer whether this reflects low potency of the skin test reagent or low level of skin sensitivity. However, the RAST results demonstrate that these skin test positive patients do indeed have IgE antibody. It appears most likely that both the need to use intradermal skin tests and the requirement for Sepharose in the RAST reflect relatively low potency of *Trichophyton* and other fungal extracts.

Traditionally, it was recognized that the different species of *Trichophyton* cross-reacted extensively but that they did not cross-react with other fungi.¹⁴ We obtained similar in vitro results with either *T. tonsurans*, *T. rubrum*, or mixed *Trichophyton* extract. Furthermore, our skin test results revealed no consistent pattern of sensitivity to other fungi in patients with positive skin tests to *Trichophyton* mixture. Although we have not carried out cross-absorption studies on sera from the minority of patients who were doubly sensitive, we think that these cases represent concordant sensitivity rather than cross-reactivity. In contrast, some patients who have strongly positive skin tests to other fungi demonstrate weak positivity to *Trichophyton*. In general, these weak reactions do not correlate with RAST results (Fig. 3). Although some of the patients who had positive RAST to *Trichophyton* were highly atopic, most were not. We have identified four patients with urticaria or angioedema and four with asthma who had no other positive skin tests. The mixed *Trichophyton* extract used here is the same extract that is widely used for testing for delayed hypersensitivity. Many of the patients and control subjects who did not demonstrate immediate skin tests developed delayed responses at 24 and 48 hours.

The finding of immediate hypersensitivity to a dermatophyte may simply reflect the widespread presence of fungal infections and their chronicity. *T. rubrum* and *T. mentagrophytes* are commonly implicated in foot, groin, and nail infections in our area, whereas *T. tonsurans* is more commonly implicated in head infections of children. The presence of IgE antibody may influence both the growth of these fungi and the local symptoms, e.g., itching of the feet or groin.^{6, 8, 9} In contrast, in patients who present with other diseases, the question arises whether the combination of *Trichophyton* allergy and absorption of fungal antigens from the skin could play a role in the other disease. This view was strongly encouraged by Sulzberger and Wise¹ in 1930 but has been largely ignored in textbooks recently. However, there are two more recent articles that report urticaria may be related to *Trichophyton* infection, and in at least one case

urticaria responded to antifungal treatment.^{2,3} We have recently reported that four of the present patients who had recurrent attacks of angioedema and/or anaphylaxis improved with antifungal treatment.¹⁰ We are not aware of any recent discussion of the role of *Trichophyton* allergy in asthma. It appeared possible in six of the present cases that *Trichophyton* could have a causative role. Improvement of asthma in two cases has coincided with treatment of the fungal infection.¹⁵ Since immediate hypersensitivity to *Trichophyton* is not restricted to patients with any one disease and occurs not infrequently in normal individuals, the association itself does not provide evidence for a causal relationship. Probably the only convincing evidence would have to come by demonstrating that antifungal treatment caused a cure or marked improvement of the associated condition. We have only occasional cases at present that have been treated in an uncontrolled fashion. We believe that our results establish the usefulness of RAST assay for confirming IgE-mediated sensitivity to this antigen and should serve to stimulate renewed interest in this area.

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REFERENCES

1. Sulzberger MB, Wise F. Urticaria and hay fever due to *Trichophyton*. JAMA 1930;99:1504.
2. Weary PE, Guerrant JL. Chronic urticaria in association with dermatophytosis: response to the administration of griseofulvin. Arch Derm 1967;95:400.
3. Waren RP, Champion RH. Urticaria. London: WB Saunders, 1974:59-62.
4. Davies RR, Ganderton MA, Savage MA. Human nail dust and precipitating antibodies to *Trichophyton rubrum* in chiropractors. Clin Allergy 1983;13:309.
5. Honbo S, Jones HE, Artis WM. Chronic dermatophyte infection: evaluation of the class-specific antibody response reactive with polysaccharide and peptide antigens derived from *Trichophyton mentagrophytes*. J Invest Dermatol 1984;82:287.
6. Hay RJ, Reid S, Talwat E, Macnamara K. Immune responses of patients with tinea imbricata. Br J Dermato 1983;108:581.
7. Masakazu A, Setsuko U, Kurakazu M, Urabe H. Purification and characterization of a new peptide antigen extracted from *Dermatophyte Mycelia*. J Invest Dermatol 1982;78:38.
8. Hay RJ, Shennan G. Antibody responses in tinea imbricata: the role of immunoglobulin E. Trans R Soc Trop Med Hyg 1984;78:653.
9. Svegaard E, Christiansen AH, Stahl D, Thomsen K. Clinical and immunological studies in chronic dermatophytosis caused by *Trichophyton rubrum*. Acta Derm Venereol (Stockh) 1984;64:495.
10. Fiocco GP, Wilkins SR, Hayden ML, Guerrant JL, Platts-Mills TAE. Anaphylaxis and severe angioedema associated with immediate hypersensitivity to *Trichophyton* infection [Abstract]. J Allergy Clin Immunol 1986;77:189.
11. Gleich GJ, Yunginger JW. Standardization of allergens. In: Rose NR, Friedman H, eds. Manual of clinical immunology. Washington, D.C.: American Society for Microbiology, 1976:575.
12. Kemeney DM, Frankland AW, Fahkri ZI, Trull AK. Allergy to castor bean in the Sudan: measurement of serum IgE and specific IgE antibodies. Clin Allergy 1981;11:463.
13. Chapman MD, Platts-Mills TAE, Gabriel M, et al. Antibody response following prolonged hyposensitization with *Dermatophagoïdes pteronyssinus* extract. Int Arch Allergy Appl Immunol 1980;61:431.
14. Tuft L. Clinical allergy. 2nd ed. Philadelphia: Lea & Febiger, 1949:571-4.
15. Platts-Mills TAE, Fiocco GP, Pollart S, Hayden ML, Jackson S, Wilkins SR. *Trichophyton* allergy in a 24-year-old man with "intrinsic" asthma. Ann Allergy 1986;56:454.

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